

Appl. No. 10/611,593
Amdt. dated September 19, 2007
Reply to Office Action of June 19, 2007

**RECEIVED
CENTRAL FAX CENTER**

SEP 19 2007

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (currently amended) A method of immobilizing a protein onto a support comprising:

- (i) attaching a ligand to a fusion protein comprising a cleavable intein under condition suitable for the cleavage of the fusion protein at the intein cleavage site to release the cleavable intein from a remaining portion of the fusion protein, and attachment of the ligand to the remaining portion of the fusion protein at the newly generated terminus of the remaining portion of the fusion protein to form a protein-ligand; and
- (ii) immobilizing the protein-ligand onto a support that is functionalized with an affinity receptor;

wherein the intein is mutated to only undergo the first step of protein splicing.

2. (original) The method according to claim 1 wherein the ligand is biotin and the affinity receptor is avidin.

3. (original) The method according to claim 2 wherein the support is glass.

4. (currently amended) The method according to claim 3 wherein the fusion protein is expressed from the expression vector pTYB1.

5. (previously presented) The method according to claim 4 wherein the ligand is cysteine-biotin and the step of attaching the ligand comprises reacting the fusion protein with cysteine-biotin.

Appl. No. 10/611,593
Amdt. dated September 19, 2007
Reply to Office Action of June 19, 2007

6. (original) The method according to claim 5 wherein the glass is functionalized with avidin by reacting the glass surface with an epoxy silane compound and reacting the resulting surface with avidin.

7. (original) The method according to claim 6 wherein the epoxy silane compound is glycidoxypyrpyl trimethoxysilane.

8. (original) The method according to claim 7 wherein avidin is streptavidin.

9. (currently amended) A method of preparing a protein array comprising the steps of

- (i) expressing a protein as a fusion protein comprising a cleavable intein and a binding domain downstream to the intein,
- (ii) contacting the expressed fusion protein with a substrate to which the binding domain binds,
- (iii) attaching a ligand to a-the expressed fusion protein comprising a cleavable intein under condition suitable for the cleavage of the fusion protein at the intein cleavage site to release the cleavable intein from a remaining portion of the fusion protein, and attachment of the ligand to the remaining portion of the fusion protein at the newly generated terminus of the remaining portion of the fusion protein to form a protein-ligand,
- (iv) immobilizing the protein-ligand onto a support that is functionalized with an affinity receptor;

wherein the intein is mutated to only undergo the first step of protein splicing.

10. (original) The method according to claim 9 wherein the ligand is biotin and the affinity receptor is avidin.

Appl. No. 10/611,593
Amdt. dated September 19, 2007
Reply to Office Action of June 19, 2007

11. (currently amended) The method according to claim 10 wherein the fusion protein is expressed from ~~the~~ expression vector pTYB1.
12. (original) The method according to claim 11 wherein the substrate is a chitin column.
13. (previously presented) The method according to claim 12 wherein the ligand is cysteine-biotin and the step of attaching the ligand comprises adding cysteine-biotin to the chitin column.
14. (original) The method according to claim 13 wherein the support is glass.
15. (original) The method according to claim 14 wherein the affinity receptor is streptavidin.
16. (original) The method according to claim 15 wherein immobilizing the protein-ligand comprises spotting the protein-ligand onto the support.
17. (withdrawn) A protein array comprising protein immobilized onto a support functionalized with an affinity receptor wherein the protein is attached to a ligand at the C-terminus by a peptide bond.
18. (withdrawn) The protein array according to claim 17 wherein the ligand is biotin and the affinity receptor is avidin.
19. (withdrawn) The array according to claim 18 wherein the support is glass.
20. (withdrawn) The protein array according to claim 19 wherein avidin is streptavidin.